Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883**
Sch. Acad. J. Biosci., 2013; 1(3):80-84

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com **DOI:** 10.36347/sajb.2013.v01i03.005

Research Article

Simultaneous Extraction and Detection of Six Fungicide Residues in Mango Fruit Followed by New Validated HPLC-UV Method

[1]Tentu. Nageswara Rao, [2]D. Sreenivasulu, [3]. B. Patrudu and [4] E.G. Sreenivasula Reddy.
[1]Department of Chemistry, Krishna University, Machilipatnam, Andhrapradesh, India.
[2]Department of Chemistry, SV University, Tirupathi, Andhrapradesh, India.
[3] Department of Chemistry, GITAM University, Hyderabad, Andhrapradesh, India.
[4] Department of Chemistry, SK University, Anantapur, Andhrapradesh, India.

Corresponding author
Tentu. Nageswara Rao
Email: tentu6581@rediffmail.com

Abstract: A simple, sensitive and inexpensive method was developed using matrix solid-phase dispersion (MSPD), together with high performance liquid chromatographic method with uv detection for determination of triazole fungicide residues (Tricyclazole, Hexaconazole, Difenoconazole) and strobilurin fungicide residues (Azoxystrobin, Trifloxystrobin, Picoxystrobin) in Mango fruit. The evaluated parameters include the type and amount of sorbent (silica gel, C18 and Activated Florisil) and the nature of eluent (ethyl acetate, dichloromethane and acetonitrile).The best results were obtained using 2.0 g of Mango fruit sample, 2.0 g of C18 as sorbent and 20ml of ethyl acetate-dichloromethane (1:1, (v/v)).The method was validated using in Mango fruit samples spiked with fungicides at different concentration levels (0.03 and 0.3 µg/mL). Average recoveries (using each concentration six replicates) ranged 90-97%, with relative standard deviations less than 2%, calibration solutions concentration in the range 0.01-2.0 µg/mL and limit of detection (LOD) and limit of quantification (LOQ) were 0.01 µg/mL and 0.03 µg/mL respectively.
Keywords: matrix solid-phase dispersion, triazole fungicides, strobilurin fungicides, HPLC-UV, LOQ, LOD.

INTRODUCTION

Fungicides are group of chemicals which are used primarily to control spoilage of crops through fungal attack. Fungicides can be divided into protectant and specific types.Protectants are the older type and includes copper and sulfur based products. They form a protective film on the plant surface and inhibit the germination of fungal spores. Specific type fungicides are so called because they act on one specific chemical reaction in the fungus. Strobilurin fungicides are one of the Specific type fungicides [1]. Strobilurins now include the world's biggest selling fungicide, azoxystrobin. By 2002 there will be six strobilurin active ingredients commercially available for agricultural use. This review describes in detail the properties of these active ingredients-their synthesis, biochemical mode of action, biokinetics, fungicidal activity, yield and quality benefits, and resistance risk, human and environmental safety. It also describes the clear technical differences that exist between these active ingredients, particularly in the areas of fungicidal activity and biokinetics. Triazole fungicides are one of the Specific type fungicides. Their invention was inspired by a group of fungicidally active natural products. The outstanding benefits they deliver are currently being utilized in a wide range of crops throughout the world. First launched in 1973, the newer triazoles, being intrinsically more active, push the sensitivity curves back to their original ED 50 values.

Various methods have been described for the determination of these pesticides, using solid-phase extraction (SPE) [2], solid-phase micro extraction (SPME) [3], Supercritical fluid extraction (SFE) [4] and matrix solid-phase dispersion (MSPD) [5-6]. However, none of the published researches to date have reported the simultaneous analysis of chemical classes such as Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobinin Mango fruit.

The matrix solid-phase dispersion (MSPD) technique was developed by Barker S.A et al. [7]. It has advantages over conventional techniques because it employs small amounts of sample and solvent, and the extraction procedure consists of only a few experimental steps. MSPD evolved from the solid-phase extraction (SPE) technique, modified for application to solid and semi-solid matrices. The MSPD procedure is based on the use of a sorbent [8], which acts as an abrasive in order to produce a modified "opening" of the solid matrix, facilitating the extraction process when using a suitable solvent for eluting the analytes. The use of MSPD for fungicide recovery depends on the solubility of the fungicide in the eluting solvent, as well as the interactions between the matrix components, sorbent and eluent.

The literature reports pertaining to the use of MSPD as an extraction technique for fungicides by MSPD is scarce. So, the present research considered six widely

used fungicides, namely Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobinin were analysis by high-performance liquid chromatography with ultraviolet detector (HPLC-UV) after subjecting to MSPD.

EXPERIMENTAL

Standards, Reagents and samples

The analytical standards of Tricyclazole (99.5%), Hexaconazole (99.2%), Difenconazole (99.2%), Azoxystrobin (99.4%), trifloxystrobin (99.2%) and picoxystrobin (98.5%), were obtained from Sigma Aldrich. Acetonitrile was purchased from Rankem, New Delhi, Analytical grade solvents, dichloromethane and ethyl acetate, were supplied from Merck Limited, Mumbai, C18-bonded silica (50 µm) from phenomenex (Torrance, CA, USA), Florisil (60-100 mesh) from fluka chemie GmbH CH-9471 Buchs, AR grade sodium sulphate from Merck Limited, Mumbai and Mango fruit was purchased from local market.

Standard stock solutions

The fungicide standard stock solutions were individually prepared in acetonitrile at a concentration level 100 µg/mL and stored in a freezer at -18°C. The stock standard solutions were used for up to 3 months. Suitable concentrations of working standards were prepared from the stock solutions by dilution using acetonitrile, immediately prior to sample preparation.

Sample preparation

Representative 2.0 g portions of Mango fruit fortified with 100 µL of working standard solution. The mixture was then gently blended in the mortar for 30 min, to assess the homogeneity of the sample. The sample was allowed to stand at room temperature for one hour, before it was kept at refrigerator condition, until analysis.

Extraction procedure

2.0 g of Mango fruit sample was weighed out and homogenized with 2.0 g of C18 –bonded silica for 5 min. The homogenized sample was transferred to an MSPD column consisting of a 20mL capacity polyethylene syringe containing 2.0 g flurosil and 2.0 g of anhydrous sodium sulfate. The elution was performed under vacuum with 20 mL of ethyl acetate-dichloromethane (1:1).The eluent was collected into a round bottom flask and evaporated to near dryness. Finally make up with 5mL of acetonitrile and analysed by HPLC-UV system.

Chromatographic separation parameters

The HPLC-UV system used, consisted shimadzu high performance liquid chromatography with LC- 20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed phase C18 analytical column of 250 mm x 4.6 mm and particle size 5 µm (PhenomenexLuna-C18) Column temperature was maintained at 40°C. The injected sample volume was 20µL. Mobile Phases A and B was Acetonitrile and 0.1% ortho phosphoric acid (75:25 (v/v)). The flow- rate used was kept at 0.7 mL/min. A detector wavelength was 235 nm. The external standard method of Calibration was used for this analysis.

Method validation

Method validation ensures analysis credibility. In this study, the parameters accuracy, precision, linearity and limits of detection (LOD) and quantification (LOQ) were considered. The accuracy of the method was determined by recovery tests, using samples spiked at concentration levels of 0.03 and 0.3 mg/kg. Linearity was determined by different known concentrations (0.01, 0.05, 0.1, 0.5, 1.0 and 2.0 µg/mL) were prepared by diluting the stock solution. The limit of detection (LOD, µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control (untreated) sample. The limit of quantification (LOQ, µg/mL) was determined as the lowest concentration of a given fungicide giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSION

Specificity

Specificity was confirmed by injecting the Mango control. There were no matrix peaks in the chromatograms to interfere with the analysis of fungicide residues shown in (Figure 1). Furthermore, the retention times of Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin were constant at 4.4 ± 0.2, 7.8 ± 0.2, 9.0 ± 0.2, 6.3 ± 0.2, 7.1 ± 0.2, and 8.4 ± 0.2, min.

Linearity

Different known concentrations of fungicides (0.01, 0.05, 0.1, 0.5, 1.0, 2.0 µg/mL) were prepared in acetonitrile by diluting the stock solution. Each solution was prepared in triplicate. Injected the standard solutions and measured the peak area. A calibration curve has been plotted of concentration of the standards injected versus area observed and the linearity of method was evaluated by analyzing six solutions. The peak areas obtained from different concentrations of fungicides were used to calculate linear regression equations. These were Y=131171.3X + 34.50, Y=104401.64X + 75.33, Y=99115.43X + 18.09, Y=128423.15 + 45.06, Y= 121224.3X + 33.12, and Y=120045.15 + 41.13, with correlation coefficients of 0.9999, 0.9998, 0.9999, 0.9998, 1.0000, and 0.9998 for Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin respectively. A calibration curve showed in (Figure 2).

Accuracy and Precision

Recovery studies were carried out at 0.03 and 0.3 µg/mL fortification levels for Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin in Mango fruit. The

recovery data and relative standard deviation values obtained by this method are summarized in Table 1. These numbers were calculated from four (6) replicate analyses of given sample (Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin) made by a single analyst on one day. The repeatability of method satisfactory (RSDs<2 %).

Detection and Quantification Limits

The limit of quantification was determined to be 0.03 µg/mL. The quantitation limit was defined as the lowest fortification level evaluated at which acceptable average recoveries (90-97%, RSD<2%) were achieved. This quantitation limit also reflects the fortification level at which an analyte peak is consistently generated at approximately 10 times the baseline noise in the chromatogram. The limit of detection was determined to be 0.01 µg/mL at a level of approximately three times the back ground of control injection around the retention time of the peak of interest.

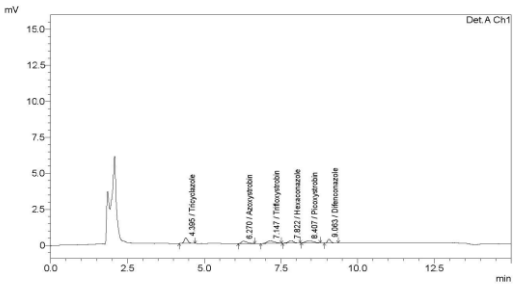

Figure 1: Representative Chromatogram at fortification level of 0.03 µg/mL

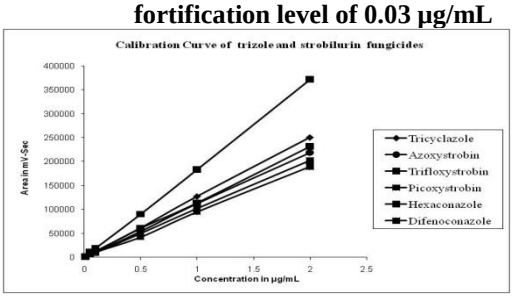

Figure 2: Representative Calibration curve of triazole and strobilurin fungicides

Table1: Recoveries of the triazole fungicides from fortified Mango control sample (n=6)

Fortification Conc. in µg/mL	Replication	Recovery (%)					
		Tricyclazole	Hexaconazole	Difenconazole	Azoxystrobin	Trifloxystrobin	Picoxystrobin
0.03	R1	88	90	89	91	87	88
	R2	90	91	88	90	90	88
	R3	91	89	89	94	90	92
	R4	89	93	90	93	88	89
	R5	89	90	91	91	91	91
	R6	90	90	93	90	90	92
	Mean	**90**	**91**	**90**	**92**	**89**	**90**
	RSD	**1.17**	**1.52**	**1.99**	**1.80**	**1.00**	**0.99**
0.3	R1	95	95	93	96	92	95
	R2	98	93	93	93	93	93
	R3	96	96	94	92	95	97
	R4	95	92	92	95	93	96
	R5	95	94	93	93	96	95
	R6	93	92	95	92	97	94
	Mean	**95**	**94**	**93**	**94**	**94**	**95**
	RSD	**1.71**	**1.74**	**1.11**	**1.76**	**0.95**	**0.94**

Storage Stability

A storage stability study was conducted at -20 ± 1°C with mango samples spiked with 0.1 µg/mL of Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin Samples were stored for a period of 30 days at this temperature. Analysed for the content of Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin before storing and at the end of storage period. The percentage dissipation observed for the above storage period was only less than 2% for Tricyclazole, Hexaconazole, Difenoconazole, Azoxystrobin, Trifloxystrobin and Picoxystrobin showing no significant loss of residues on storage. The results are presented in table 2.

Table2. Storage stability Details (n=6)

Fortification Conc. in µg/mL	Storage Period in Days	Replication	Recovery (%)					
			Tricyclazole	Hexaconazole	Difenconazole	Azoxystrobin	Trifloxystrobin	Picoxystrobin
0.1	0	R1	87	88	86	86	88	88
		R2	90	88	88	88	88	87
		R3	90	92	89	90	89	90
		R4	88	89	88	90	87	89
		R5	91	91	87	88	87	89
		R6	90	92	90	90	89	90
		Mean	**89**	**90**	**89**	**89**	**88**	**89**
		RSD	**1.69**	**2.11**	**1.58**	**1.84**	**1.19**	**2.14**
	30	R1	92	95	96	94	90	93
		R2	93	93	95	97	93	95
		R3	95	97	92	95	92	96
		R4	93	96	93	95	91	96
		R5	96	95	94	96	94	93
		R6	**97**	**94**	**96**	**94**	**96**	**92**
		Mean	**94**	**95**	**94**	**95**	**93**	**94**
		RSD	**2.09**	**1.48**	**1.73**	**1.23**	**2.13**	**1.50**

CONCLUSION

This paper describes a fast, simple sensitive analytical method based on MSPD-HPLC-UV simultaneous determination of strobilurin and triazole fungicide residues in Mango fruit. The MSPD extraction procedure is very simple and requires no sample preparation or pre-treatment, providing adequate clean-up of the matrix. Whole mango extracts are very clean, with no interfering peaks at the retention time of the target compounds, indicating good selectivity of the proposed method.

The mobile phase Acetonitrile and 0.1% ortho phosphoric acid showed good separation and resolution and the analysis time required for the chromatographic determination of the strobilurin and triazole fungicides is very short (around 15 min for a chromatographic run).

Satisfactory validation parameters such as linearity, recovery, precision and very low limits were obtained and according to the SANCO guidelines [9]. For all of the Triazole fungicides the sensitivity of the method was good enough to ensure reliable determination levels lower than the respective MRLs. Therefore, the proposed analytical procedure could satisfactorily be useful for regular monitoring of Strobilurin fungicide residues on a large number of fruit samples.

ACKNOWLEDGEMENT

The authors are thankful to the CH. Nagaraj, IICAT for his keen interest and help.

REFERENCES

1. Bartlett DW, Clough JM, Godwin JR, Hall AA, Mick Hamer, Parr-Dobrzansk B; The strobilurin fungicides. Pest Management Science, 2002; 58 (7): 649-662.
2. Giraud D, Ventura A, Camel V, Bermond A and Arpino P; Determination of traces of pesticides in water by solid-phase extraction and liquid chromatography-ionspray mass spectrometry. J Chromatogr . A., 1997; 777 (1):115-25.
3. Choudhury TK, Gerhardt KO and Mawhinney TP; Solid-Phase micro extraction of nitrogen and phosphorus containing pesticides from water and gas chromatographic analysis. Environ. Sci. Technol, 1996; 30(11): 3259-3265.
4. Zuin VG, Yariwake JH and Langas FM; Analysis of pesticide residues in Brazilian plants. Braz. Chem. Soc, 2003; 14: 304-309.
5. Aquino A; Souza MRR; Maciel STA; Alexandre MR and Navickiene S; Multiclass MSPD Method for Pesticide Determination in dehydrated Hyptis pectinata medicinal plant by GC-MS. Braz. Chem. Soc., 2011; 22 (8).
6. Barker SA, Long AR and Short C.R: Isolation of Drug residues from tissues by solid phase dispersion. J. Chromatogr. A, 1989; 475 (2): 363-361.
7. SA Barker, AR Long, and CR Short; Isolation of drug residues from tissues by solid phase dispersion. J. Chromatogr. A, 1989; 475: 353–361.
8. Lehotay SJ; Analysis of pesticide residues in mixed fruit and vegetable extracts by direct sample introduction/ Gas Chromatography/ Tandem Mass Spectrometry. Journal of AOAC International, 2000; 83 (3): 680-697.

9. SANCO Guidelines: Method validation and quality control procedures for pesticide residues analysis in food and feed. Document NO. 2009; SANCO/10684/2009.